

REMARKS

Reconsideration and continuing examination of the above-identified application is respectfully requested in view of the amendments above and the discussion that follows.

Claims 1-9, 12-33, 35-38 and 42-78 are in the case and are before the Examiner.

I. The Action

A. Rejections Under 35 USC §112,

First Paragraph

It is noted with appreciation that the prior rejection based on the First Paragraph of Section 112 has been withdrawn.

B. Rejection Under 35 USC §103(a):

Pumpens In View Of Zlotnick

Claims 1-9, 15, 16, 18-26, 30-33, 35, 38, 42-58, 63-75, 77 and 78 have been rejected as allegedly obvious over the disclosures of Pumpens (1995) in view of Zlotnick. This rejection is again respectfully traversed as discussed hereinbelow.

The present Action has reiterated the rejection from the previous Action, and has again failed to properly embrace the importance of the teachings of workers at least ordinary skill in this art, Ulrich et al., Newman et al. and Lachmann et al. Those researchers worked in this field, knew of and cited Zlotnick's work and disregarded it for dealing with particle stability. Disregarding those real workers of at least ordinary skill, the Actions have grasped and held on to the straw of an imaginary combination of the Pumpens and Zlotnick teachings that the real workers rejected or did not contemplate. This failure to appreciate the true teachings of the art to forward-looking

workers of ordinary skill is inappropriate and should be withdrawn. The previously filed arguments are hereby incorporated by reference into this reply.

Second, and looked at from another direction, it is respectfully submitted that the teachings of Pumpens and those of Zlotnick are incompatible with each other and so cannot properly be combined in the manner suggested in the Action.

In particular, no construct of Zlotnick contained an added, heterologous epitope as is claimed here. Zlotnick neither teaches nor suggests anything about the effect of a C-terminal cysteine on a truncated HBc molecule that retains its internal cysteines at internal positions 48, 61 and 107, nor on such a molecule that has any inserted sequence.

Pumpens teaches at page 67, lower left, that "capsids formed by C-terminally truncated HBc monomers are less stable than the corresponding full-length protein particles" and that foreign insertions internal to the sequence "exert a stabilizing effect on chimeric HBc delta derivatives". Thus, Pumpens teaches insertion of such sequences to enhance stability, which is an entirely different approach to Zlotnick and incompatible with the latter.

We ask again, why would a skilled worker combine the teachings of Pumpens and Zlotnick when Pumpens teaches that one can gain stability in C-terminally truncated chimeras by having internal insertions, and Zlotnick allegedly says one can gain particle stability by adding a terminal Cys? The answer is that a skilled worker looking forward and without the benefit of this application would not make the combination. As such, it is submitted that the two teachings are not appropriately combined in the Action and the rejection should be withdrawn.

If the two teachings were combined, because there is no independent teaching of what should be kept and what should not, nor of where any part should be placed, the structure that one gets is that of an HBc molecule that has no internal cysteines, has a peptide insert at an unspecified location in the sequence, is truncated at the C-terminus and has a C-terminal cysteine. That construct is not claimed. As such, this rejection should again be withdrawn.

Third, the Action has placed great emphasis on the results reported by Zlotnick regarding the effect of the C-terminal cysteine residue present in the so-called Cp\*150 construct and how those effects would suggest the claimed invention to a worker of ordinary skill in the art. As already noted, it is believed that the Zlotnick teachings are not combinable with those of Pumpens, nor with any other teachings because they are taken out of context and have been misinterpreted.

First, the premise that Zlotnick teaches that C-terminal cysteine can stabilize an HBc chimera particle as recited in the claims here cannot be agreed with. This premise is inconsistent with the statements and data provided therein by Zlotnick.

For example, Zlotnick explicitly states: "[p]urified Cp\*149 and Cp\*150 assemble into capsids under the same conditions as other constructs, with or without DTT. These capsids [i.e., particles] were *indistinguishable* (emphasis added) by negative staining electron microscopy and sedimentation on sucrose gradients." (See page 9558, column 1, paragraph 1, Results and Discussion section.) As a second example, Zlotnick reports: "[a]t a resolution of  $\approx 20\text{\AA}$ , the outer surface of the Au11-labeled [monomaleimidyl-undecagold-labeled]

Cp\*150 capsid is *indistinguishable* (emphasis added) from those of unlabeled Cp147 and Cp183 capsids, (cf. Fig. 4 Top)." (See page 9558, column 2, paragraph 1.) These facts would lead one skilled in the art to conclude that C-terminal cysteines are not important for HBcA capsid formation or stability.

Examination of Fig. 2a of Zlotnick shows that the greatest mass there shown for reduced and non-reduced protein corresponded to a little more than that of the 31 kDa molecular weight standard. That molecular weight is that expected for a dimer of two strands of 150 residues (150 residues X 109 average molecular wt/residue = 16.35 kDa). The reported results in that figure therefore relate only to dimers and monomers. The present claims recite stability of the particles assembled from those monomers and dimers. As such, a disclosure concerning the stability or lack thereof of dimers or monomers neither teaches nor suggests anything of relevance to the claimed subject matter whether taken alone or with any other disclosure. Still further, it is not logical to conclude that large molecular weight, structurally folded, dense capsid molecules that contain 90 or 120 dimers would behave identically to their constituent dimers. One of skill in the art would recognize this.

Still further, nothing in Zlotnick has shown that capsids behave like dimers. For example, Zlotnick states: "[a]ssembled capsids of reduced Cp\*150 did not react with Au11. Apparently, the C-terminal cysteine is inaccessible. Accordingly, Cp\*150 was labeled with Au11 at neutral pH and low ionic strength, where the sample is mainly free dimers." (See page 9558, column 1, paragraph 3.) Therefore, it is improper to conclude that capsids behave like dimers as was done in the Action in reference to Figure 2 of Zlotnick.

Importantly, it further appears that in Zlotnick's Figure 2, at least two crucial controls are lacking. One missing control is a sequence of the truncated 150-mer with the C-terminal cysteine replaced by an alternative amino acid. Without this crucial control, one of skill in the art would reasonably conclude that the polymerization of Cp\*150 was due to the increase in length of the peptide from 149 amino acids to 150 amino acids. This is a perfectly plausible conclusion given Zlotnick's quoted statement "[p]urified Cp\*149 and Cp\*150 assemble into capsids under the same conditions as other constructs, with or without DTT. These capsids [i.e., particles] were *indistinguishable* (emphasis added) ...". In fact, this is the *only* difference between Cp\*149 and Cp\*150. Zlotnick did not show otherwise.

This conclusion is even more plausible given the fact the addition of gold at the C-terminus also promotes aggregation. Zlotnick states: "[t]hus it appears that modification of Cp\*150 with Aull promotes polymerization. The Aull has a single reactive maleimide group and cannot crosslink proteins." (See page 9558, column 1, paragraph 3.) Also, Zlotnick states: "[o]ther observations imply that the C termini also may influence assembly in more subtle ways. For instance binding Aull to Cp\*150 induces assembly, though Aull cannot crosslink subunits, nor, because of its organic shell, coordinate C-terminal cysteines." (See page 9540, column 2, paragraph 1.)

In that same paragraph, while relating to the gold-labeled mutants, Zlotnick further goes on to hypothesize that changes in the molecular surface near the C-terminus may stimulate the assembly process. Therefore, one of skill in the art would reasonably conclude from these statements that

Zlotnick teaches that dimer formation is induced by merely increasing length of the mutant protein, since aggregation also occurs in the absence of free cysteine at the C-terminus of the protein.

The second control that is lacking in Zlotnick is that of Cp\*150 with the internal cysteines at positions 48, 61 and 107 in place. Without this second crucial control, one of skill in the art could not conclude that any alleged increase in capsid stability of Fig. 2b was due to terminal cysteines as proposed in the Action. The alleged increase in dimerization shown in Fig. 2a could also be due to the removal of those amino acids, which might have profound effects on the folding and stability of the molecule as is shown in the present application. Zlotnick does not provide any data nor does he discuss any proteins with internal cysteines.

The only C-terminal mutant protein that Zlotnick shows in the polyacrylamide gel and size exclusion chromatography of Figure 2 does not contain any internal cysteines, unlike the mutants of the present application. One of ordinary skill in the art would recognize that the presence or absence of these moieties would undoubtedly change the structure, folding and intramolecular binding of the mutant protein. Zlotnick does not take into account this critical feature in the design of his experiments, and neither has the Action in its assertions about the relevance of the Zlotnick disclosures.

Furthermore, upon careful scrutiny of Zlotnick's cited gel (Fig. 2a), one can see in lane 7 that there is a band at the lower edge of the gel near the spot of 14.4 kDa standard that clearly indicates that some of the Cp\*150 sample has either failed to associate into a dimer or has readily dissociated at pH 9.5. There is a similar band at pH 7.5. A recently obtained

sharper, color copy of the Zlotnick paper is attached for the Examiner's convenience to better see those disclosures.

The size exclusion chromatography graph in Fig. 2b is also not informative. No standards were shown. Without some standard to measure by, one cannot reliably state what the depicted plots show.

The disclosure in Fig. 2b appears to compare the stability of particles Cp\*150 particles in oxidized or reduced form and Cp\*149 particles after exposure to 3.5 molar urea. The figure has no data showing the stability of particles assembled from a chimeric protein having a structure that includes internal cysteine residues present at positions 48, 61 and 107 as are present in the claimed subject matter, nor for another 150 residue HBc with no cysteines at all.

That figure also has no data showing the stability of particles containing "one or more peptide-bonded heterologous epitopes at the N-terminus, or in the HBc immunogenic loop or a heterologous linker residue for a conjugated epitope present in the HBc immunodominant loop" as is also present in the claimed subject matter. Because of the lack of proper control data, it is submitted that the data in Fig. 2b also neither teach nor suggest anything of relevance to the worker of ordinary skill in this art regarding the claimed subject matter whether taken alone or with any other disclosure.

The Action asserted in Paragraphs 10 and 11 that the amendment to the independent claims "did not constitute a structural or functional limitation to the alleged HBc chimera . . ." as that amendment related to a method of measurement. It is submitted that the recited assay technique along with the previous recitation of enhanced stability together constitute a

functional limitation by reciting how one determines enhanced stability of the formed particles.

The results shown in Zlotnick's Fig. 2a indicate that the protein of the cysteine-containing chimeres polymerized to dimers at pH 9.5 were less pure than that polymerized at pH 7.5. It is submitted that Zlotnick has no teaching related particle stability in the form of protein degradation, as compared to dimer stability. The claims were amended to clarify that the stability recited relates to the particles, and as such constitute a functional limitation. It is thus again submitted that this basis for rejection should be withdrawn.

C. Second Rejection Under 35 USC §103(a)

Pumpens In View Of Zlotnick and Thornton

Claims 12-14, 17, 27-29, 36, 37, 59-62 and 76 were again rejected as allegedly obvious from the combined disclosures of Pumpens in view of Zlotnick as above, and further in view of Thornton et al. US Patent No. 5,143,726. The Thornton et al. patent is relied-on for its disclosures of linking a polypeptide to core via "an amino acid side chain on the core molecule . . ." (Action, page 7). This rejection is respectfully traversed as discussed below.

The deficiencies of the combined teachings of Pumpens and Zlotnick from above and the previous discussions are hereby repeated. In addition, the presently rejected claims recite directly or indirectly that the chimeric molecule "contains a heterologous linker residue for a conjugated epitope". That linker residue can be at the N-terminus, in the of the HBC molecule that include a peptide-bonded heterologous epitope or a heterologous linker residue for a conjugated epitope present in



the HbC immunodominant loop, or at the C-terminus (see for example, claim 18).

On the other hand, Thornton teaches use of amino acid side chains that are endogenously present. Thornton also teaches that endogenous residues can be modified, but fails to identify where the modifications should go and fails to provide the specificity required if such a disclosure were to have been made in the present application. As such, the Thornton disclosure is not enabling for the use put to it by the Action because it not only does not put this claimed invention into the hands of the public, it does nothing to assist the public in grasping this invention. As a consequence, Thornton is inappropriately relied-on here and this rejection should be withdrawn. [*In re Hoeksema*, 399 F.2d, 269 (CCPA 1968); *In re Brown*, 477 F.2d 951; *In re LeGrice*, 301 F.2d 929, (C.C.P.A. 1962).]

That Pumpens teaches an amino acid sequence of hundreds of residues can successfully be inserted into the loop does not teach or suggest that one can add a single residue and successfully couple an oligopeptide or oligosaccharide, for example, to an added residue in the same region of a HbC chimera. Zlotnick has no suggestion of even adding a sequence into the HbC backbone, let alone adding a sequence that is substantially perpendicular to the backbone, nor does Zlotnick suggest what might happen to such a chimera protein on standing in an aqueous buffer for about two weeks. It is again submitted that this rejection should be withdrawn.

#### D. Summary

Each of the bases for rejection has been dealt with and overcome or otherwise made moot.

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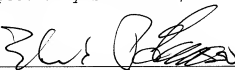
It is therefore believed that this application is in condition for allowance of all of the pending claims. An early notice to that effect is earnestly solicited.

No further fee or petition is believed to be necessary. However, should any further fee be needed, please charge our Deposit Account No. 23-0920, and deem this paper to be the required petition.

The Examiner is requested to phone the undersigned should any questions arise that can be dealt with over the phone to expedite this prosecution.

Respectfully submitted,

By



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Enclosures

Petition and Fee  
RCE and fee  
Color copy of Zlotnick paper